

Determination of maternal lipid peroxidation and antioxidant activities in term and preterm birth in different weeks

Oxidative stress in term and preterm birth

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Abstract

Aim: It has been suggested that oxidative stress may be associated with various pregnancy complications, including preterm birth (PTB). However, the role of oxidative stress in preterm births and its effects on the pregnancy process are not conclusive.

Material and Method: In this study, oxidative stress parameters were investigated in maternal blood samples who delivered at preterm and term. One hundred twelve mothers (<37 gestational weeks) diagnosed with preterm delivery were included in the study as a patient group. Also, sixty-four women who delivered at term as a control group were included in the study. Serum antioxidant enzymes (CAT (Catalase), SOD (Superoxide dismutase), GSHPx (Glutathione peroxidase), GSH (Glutathione) and MDA (Malondialdehyde) levels were determined spectrophotometrically.

Results: Serum antioxidant activity was lower in the preterm groups than in the control group. Also, serum lipid peroxidation levels were higher in the preterm groups than the control group ($p < 0.05$).

Discussion: Our findings show that women with preterm birth have higher levels of oxidative stress. These results suggest that oxidative stress is associated with preterm labor. However, it is still unclear whether oxidative stress is a cause or a result of preterm birth.

Keywords

Preterm birth; Antioxidants; Lipid peroxidation; Oxidative stress

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Introduction

Preterm birth (PTB) is defined as birth that occurs before 37 weeks of gestational age. Worldwide, 15 million babies are born prematurely each year. Preterm birth is a major cause of neonatal morbidity and mortality, especially in developing countries. [1]

Preterm births can be classified into three groups as follows: spontaneous preterm delivery (40-50%), preterm premature membrane rupture (25-40%) and obstetric preterm births (20-25%). There are also four degrees of prematurity: extreme small preterm (before 28 weeks), very small preterm (28-31 weeks), mild preterm (32-33 weeks) and moderate preterm (34-36 weeks) [2]. Factors contributing to the establishment of PTB include inflammation or infection, young or advanced maternal age, multiple pregnancies, low maternal body mass index and lifestyle (excessive exercise, alcohol, smoking) [3-4].

Oxidative stress is characterized by excessive production of reactive oxygen species (ROS) with inadequate antioxidant defense mechanisms. Increased oxidative stress results in damages of DNA, proteins and lipids. Antioxidants protect DNA, enzymes, proteins and membrane phospholipids by neutralizing free radicals. [5]. The antioxidant defense system mainly contains enzymes such as superoxide dismutase, glutathione peroxidase, catalase and antioxidant molecules such as GSH. Superoxide dismutase (EC1.15.1.1) is an antioxidant enzyme that catalyzes the decomposition of high reactive superoxide anion into O₂ and H₂O₂. [6]. CAT and GPX play an important role in the detoxification of H₂O₂. CAT (EC1.11.1.6) reacts with H₂O₂ and hydrogen donors with peroxidase activity to form water and molecular oxygen. Thus, protection is provided against H₂O₂ formed inside the cells. GPX (EC1.11.1.19) glutathione peroxidase is an intracellular selenoprotein enzyme that reduces H₂O₂ using GSH. Glutathione is an intracellular cysteine tripeptide and is primarily present in cells in reduced form (GSH) or in oxidized form (GSSG) [7]. Lipid peroxidation occurs as a result of damage caused by free radicals and produces secondary products containing aldehydes such as malondialdehyde (MDA) [8]. Elevated MDA levels have been reported to be an important indicator of oxidative stress [9]. Different results have been reported in previous studies regarding the relationship between oxidative stress and premature birth. In some of these studies, no difference was found between preterm and term groups in terms of MDA levels [10], while in others, MDA levels were found to be higher in preterm births [11]. Also, some studies have reported that antioxidant levels are higher or lower in women with preterm birth [11,12].

The present study was designed to determine serum lipid peroxidation (MDA) and antioxidant (SOD, CAT, GSHPx and GSH) activity in preterm and full-term deliveries.

Material and Methods

Subjects

In this study, 112 cases (<37 gestational weeks) diagnosed as preterm delivery in Obstetrics and Gynecology Department Risky Pregnancy Unit of the Van Regional Training and Research Hospital were included. In addition, 64 healthy pregnant women were included in the study, as a control group, who were

followed up in the antenatal unit without any complications of pregnancy.

The pregnant women included in the study were divided into 3 groups according to the gestational week. The first group consisted of healthy pregnant women with a mean gestational age of 39.4 ± 1.89 weeks. The second and third groups consisted of women who had preterm labor. The first preterm group consisted of 56 women and the mean gestational age was 29.3 ± 3.45. The second preterm group consisted of 56 women with a gestational age of 35.3 ± 1.67 weeks. The gestational weeks of the subjects included in the study were calculated based on their last menstrual period and/or based on ultrasound examination performed in the first trimester. Pregnant women with systemic diseases such as gestational diabetes, preeclampsia, thyroid dysfunction, chronic hypertension or membrane rupture, placental pathology, intrauterine growth retardation, fetal abnormalities or fetal distress were excluded from the study. Also, smokers were excluded from both groups. All patients were informed about the details of the study and written consent was obtained. All procedures were performed in accordance with the ethical standards of the Declaration of Helsinki. Permission was obtained from the Van Regional Training and Research Hospital Ethics Committee for Non-interventional Clinical Researches.

The diagnosis of preterm delivery was made in the presence of uterine contractions before the 37th week of pregnancy with intact amniotic membranes. Uterine contractions occur at least twice every 10 minutes and cause cervical changes (cervical dilatation > 2 cm and effacement > 50%) and did not stop, although hydration and bed rest were considered criteria for the diagnosis of preterm delivery. All patients included in the study underwent a routine medical and obstetric examination. Blood and urine samples were obtained for hemogram, ALT, AST, fibrinogen, complete urinalysis, blood group and urine culture. All participants had singleton pregnancies and delivered vaginally without anesthesia.

Biochemical analysis

Biochemical analyzes were performed in the Chemistry Department Biochemistry Laboratory of Yuzuncu Yil University. Blood samples (10 ml) were obtained from all participants during delivery and put into plain tubes. Blood samples were centrifuged at 5000 rpm for 10 minutes to obtain serum. Serum samples were placed in polypropylene tubes and kept in the freezer at -20 °C until the working day.

MDA was estimated by measuring TBARS (thiobarbituric acid-reactive substances) in serum samples according to a modified method of Jentzsch et al. [13]. First, 0.2 ml of serum was added to the reaction mixture containing 1 ml of 1% ortho-phosphoric acid, 0.25 ml alkaline solution of thiobarbituric acid-TBA (final volume 2.0 ml), followed by heating for 45 min at 95 °C. The results were expressed as mmol MDA per liter of serum.

Superoxide dismutase, catalase, glutathione peroxidase and glutathione activities were also measured in serum samples as markers of the antioxidant system. CAT activity: Serum CAT activity was measured using H₂O₂ as the substrate. The change of H₂O₂ levels was monitored at 240 nm for 5 min using a spectrophotometer, and enzyme activity was expressed in units per liter (U/L) [14]. Serum SOD activity was measured

in accordance with the method of Sun et al. [15]. The activity of GSHPx enzyme was measured according to Paglia and Valentine [16]. The GSHPx enzyme catalyzes the oxidation of glutathione. When the oxidized glutathione is reduced, NADPH (nicotinamide adenine dinucleotide phosphate) is oxidized and turned into NADP. This change was observed at 340-nm wave and the activation of GSHPx was measured. The results were expressed as units per liter (EU/L) for serum. The GSH level was measured spectrophotometrically at 412 nm by a glutathione disulfide reductase recycling method at room temperature [17].

Statistical Analysis

Descriptive statistical data for the continuous variables were expressed as mean \pm SD (Standard deviation). ANOVA (One Way Analysis of Variance), was used for normal distribution conditions and Kruskal-Wallis test statistic was used for cases where a normal distribution condition was not provided. The results were considered to be statistically significant when $p < 0.05$. The data were analyzed using the SPSS 19 software (SPSS Inc., Chicago, IL, USA).

Results

There was no statistical difference between the study and control groups in terms of maternal age, gravida (number of pregnancy) and number of births (parity) (Table 1). However, there was a difference between the groups in terms of the gestational week. While the mean gestational week was 29.3 ± 3.45 in the preterm group I, the mean gestational week was 35.3 ± 1.67 in the preterm group II. The mean gestational week in the control group was 39.4 ± 1.89 . Systolic and diastolic blood pressure values of all participants were within normal limits. In addition, blood ALT (Alanine transaminase), AST (Aspartate transaminase), glucose, hemoglobin, fibrinogen, platelet and protein content in spot urine were within normal limits (Table 1).

Table 1. Characteristics and some biochemical data of term (control) and preterm groups

	Term (control) group (n=64)	Preterm group I (n=56)	Preterm group II (n=56)	p
Maternal age	27.5 \pm 5.60	26.7 \pm 6.32	27.1 \pm 6.44	0.427
Gestational week	39.4 \pm 1.89	29.3 \pm 3.45	35.3 \pm 1.67	0.001
Gravida	4.11 \pm 1.58	3.62 \pm 1.13	4.01 \pm 1.65	0.347
Parity	2.10 \pm 1.55	1.78 \pm 1.32	2.04 \pm 1.41	0.646
Systolic BP(mm/Hg)	122 \pm 12.34	127.4 \pm 7.33	124.6 \pm 10.81	0.541
Diastolic BP(mm/Hg)	82.3 \pm 5.69	85.7 \pm 8.22	86.8 \pm 6.55	0.231
Glucose (mg/dl)	86.1 \pm 4.7	94.1 \pm 4.8	89.6 \pm 5.3	0.546
Haemoglobin (g/dl)	12.3 \pm 1.87	12.5 \pm 1.67	11.9 \pm 1.35	0.685
AST (U/L)	28.8 \pm 8.4	25.1 \pm 3.1	29.3 \pm 7.6	0.154
ALT(U/L)	23.4 \pm 3.1	20.4 \pm 5.3	26.3 \pm 7.2	0.480
Fibrinogen (mg/dl)	242.12 \pm 43	254.23 \pm 34	234.10 \pm 66	0.532
Protein in spot urine (mg/dl)	11.1 \pm 3.22	10.3 \pm 4.01	12.6 \pm 5.5	0.527
Platelets (10^9 /uL)	310.11 \pm 22.9	322.14 \pm 32.6	285.34 \pm 57.3	0.543

Values are means \pm standard deviation, ($p < 0.05$).

Table 2 shows the serum antioxidant enzymes and MDA levels of the participants. There was no difference between the preterm I and II groups in terms of SOD activity, but SOD activity was found to be lower in both preterm groups than the control group.

Table 2. Serum antioxidant and malondialdehyde levels in term and preterm groups

	Term group (n=64)	Preterm group I (n=56)	Preterm group II (n=56)	p
SOD (U/ml)	7.821 \pm 0.234	2.513 \pm 0.459	2.546 \pm 0.311	0.001
CAT (U/L)	0.207 \pm 0.003	0.065 \pm 0.009	0.029 \pm 0.004	0.001
GSH (mmol/ml)	0.272 \pm 0.016	0.080 \pm 0.005	0.056 \pm 0.002	0.001
GSHPx (U/L)	14.101 \pm 0.603	5.684 \pm 0.442	7.259 \pm 0.140	0.001
MDA (mmol/L)	1.064 \pm 0.017	2.601 \pm 0.071	1.118 \pm 0.006	0.001

* It implies the statistical significance between patients and control group ($p < 0.05$). Values are means \pm standard deviation

CAT activity was lower in the preterm II group than the preterm I group, whereas CAT activity in both preterm groups was lower than the control group. GSH and GSHPx activity were lower in both preterm groups compared to the term group. MDA levels were higher in preterm groups compared to the term group. In addition, according to the correlation analysis, there was no relationship between antioxidant activities and MDA level.

Discussion

In this study, serum antioxidant enzymes CAT, SOD, GSH, GSHPx and MDA levels, which are the end product of lipid peroxidation, were investigated in preterm and term delivery. MDA levels were higher in preterm groups than in the term group. On the other hand, antioxidant enzyme activity was lower in preterm groups compared to the term group.

Premature births are the leading cause of neonatal deaths occurring every year around the world. It is estimated that 11% of all births result in premature delivery [1]. Previous studies have reported that oxidative stress during pregnancy may be associated with preterm delivery. [18]. Oxidative stress, defined as an imbalance between antioxidants and reactive oxygen species. In fact, when reactive oxygen species, produced in biological systems are at low levels, it is necessary for some cellular functions such as cell division, inflammation, and immunity [19]. However, excessive free radical production can cause cellular damage by exceeding a biological system's ability to detoxify them [20]. High levels of oxidative stress in pregnant women may lead to placental dysfunction or other damages leading to preterm delivery [21].

The role of oxidative stress and antioxidants in preterm and term delivery is not fully understood, but some mechanisms have been proposed. First, it has been suggested that increased reactive oxygen species may serve as a precursor for inflammatory responses that can initiate premature labor and damage cervical stroma or collagen in fetal membranes [22]. Secondly, it may cause dysfunctional placenta localization by reducing the spiral arteriole invasion of the myometrial wall in the early period of pregnancy [23].

Balancing oxidative stress can be accomplished with an antioxidant defense system consisting of enzymes such as catalase, superoxide dismutase, glutathione peroxidase, and non-enzymatic antioxidant such as glutathione. Antioxidants protect cells by inhibiting oxidation reactions, and thus play an important role in maintaining cellular function in normal pregnancy [5].

Different results have been obtained in previous studies

on the relationship between preterm delivery and oxidative stress. Cinkaya et al. [12] found that total antioxidant levels were lower in women with preterm delivery compared to uncomplicated pregnant women at similar gestational week. Also, Joshi et al. [11] found that MDA levels were higher in preterm delivery compared to term delivery, and found that this correlates with samples from neonatal cord blood. In the same study, maternal blood vitamin C levels were found to be higher in the preterm group than in the term group. However, in some studies, there was no difference between preterm and term deliveries in terms of antioxidant and lipid peroxidation levels. In a study, women of similar age were divided into 3 groups: preterm, term, and non-pregnant healthy controls. As a result of this study, it was determined that GSH and GSHPx activities did not differ between term and preterm groups [24]. In another study, maternal and neonatal cord blood samples were examined for oxidative stress markers and micronutrients, and it was found that there was no difference in MDA levels and micronutrient levels (β -Carotene, alpha-Tocopherol, lutein) in preterm and term delivery [10]. Another study investigated the association between pregnancy complications and maternal oxidative stress. They compared pregnant women with normal pregnancy and those with complications during pregnancy (preeclampsia, preterm birth, low birth weight). When they compared, it was found that plasma total antioxidant capacity (TAC) and erythrocyte GSHPx and SOD activities did not differ between the two groups. However, in the same study, plasma 8-isoprostane (oxidative stress marker) levels were found to be significantly higher in pregnant women who subsequently developed preeclampsia or SGA (small for gestational age infant) compared with normal pregnancies [25].

Our study has some limitations. Blood samples were taken only from the mother at birth, cord blood samples from newborns were not taken. Therefore, we could not evaluate whether the oxidative stress in the mother affects the baby. In addition, we could not control the effects of maternal characteristics such as body mass index (BMI), exercise and nutritional status.

In conclusion, our findings show that women with preterm birth have higher levels of oxidative stress. These results suggest that oxidative stress is associated with preterm labor. Although the effects and mechanisms of antioxidants on the pregnancy process are not yet fully understood, it should be taken into account when diagnosing treating preterm delivery. On the other hand, the use and effectiveness of antioxidant treatments in risky pregnancy still remain a controversial issue. Also it is still unclear whether oxidative stress is a cause or a result of PTB.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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